Pre-Synopsis

Title of the research work

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Under the Guidance: Dr.Babu R L

"Molecular diversity and DNA Barcoding of fruit fly *Bactrocera zonata* (Tepritidae:Diptera) in Northern Karnataka based on cox1 gene sequences"

Introduction:

Fruit flies are the major insect pests of horticulture crops across the globe with high genetic diversity and taxonomic complexity. There are nearly 5000 species described under 500 family of Tephritidae with re-categorization occurring constantly with deeper taxonomic investigations. Fruit flies are divided into four main groups *viz*., *Ceratitis*, *Bactrocera*, *Anastrepha* and *Rhagoletis*. In genus *Bactrocera* there are about 500 species arranged in 28 subgenus. The peach fruit fly, *Bactrocera zonata* (Saunders) is one of the most devastating species in the genus Bactrocera and a notorious polyphagous fruit fly pest attacking many commercially important fruit crops in many parts of the world including India (1). Being multivoltine and highly polyphagous, *B. zonata* can infest fleshy fruits of more than 50 species of cultivated and wild plants (4) *viz*., mango, fig, guava, peach, apple, citrus, papaya, Ber, saporta, orange, Lemon, Plum and other fruit crops.

B. zonata is originated from South and South East Asia and spreads throughout Asia *viz*., Bangladesh, Bhutan, India, Iran, Laos, Myanmar, Nepal, Oman, Pakistan, Sri Lanka, United Arab Emirates, Thailand, Vietnam and Yemen. But it has invaded several countries and is regarded as a serious threat to Middle East (Saudi Arabia), North Africa (Egypt ,Libya, Mauritius and Reunion), Australia and to a lesser extent in southern Europe (5). It has been established in Egypt since1998 and has already ecologically displaced *Ceratitis capitata*.

In India *B. zonata* was first recorded in Bengal (India) by Saunders during 1842 (6). Fruit flies have been identified as one of the ten most serious problems of agriculture in India, because of their polyphagous nature and cause a huge economic loss to fruits and vegetables, which varies from 25 - 100 per cent with loss of Rs 2945 crore per annum (Sumathi *et al*., 2019). In certain areas of north India and Pakistan, it has been more notorious than *B. dorsalis.*

Owing to their enormous damage potential, faster acclimatization and adaptability, many fruit fly species have been recommended for domestic and international quarantine to restrict their entry into new habitats. Information regarding accurate, faster identification, genetic diversity and genetic structure of *B. zonata* is essential for the planning and executing appropriate management

strategies. The molecular diversity of *B. zonata* have remained relatively unexplored in India particularly in Karnataka. Hence, I would like to make an attempt to investigate survey, population dynamics of *B. zonata* and molecular diversity of *B. zonata* in cox 1 and nad 1 regions of mitocondrian DNA, further comparative genomics will be carried out.

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Objectives

1) Survey for the collection of fruit fly *B. zonata* from different parts of Northern Karnataka
2) Studying the population dynamics of fruit fly *B. zonata* in the selected districts of Northern Karnataka
3) Studying the molecular diversity of fruit fly *B. zonata* populations in the cox 1 region of mitochondrial DNA
4) To study the DNA Barcoding of fruit fly *B. zonata*

Methodology

1) Survey for the collection of fruit fly *B. zonata* from different parts of Northern Karnataka

Methyl eugenol traps will be installed in different districts of northern Karnataka and a month later fruit flies trapped in the container will be collected in paper bags, will be brought to laboratory and kept in Refrigerator for further use. The date and place of collection of fruit flies will be is labeled on the paper bags. The collected fruit flies will be used for DNA extraction studies.

2) Studying the population dynamics of fruit fly *B. zonata* in the selected districts of Northern Karnataka

Simultaneously fruit fly traps will be installed fruit crops in Vijayapura, Riachur and Yadgiri districts to assess the species complexity and population dynamics of fruit fly *B. zonata*. This study will be continued for two years and lures will be changed in once in three months (Sumathi et al., 2019). Every month fruit flies trapped in the trap will be collected in paper bags, brought to laboratory. The fruit flies will be identified based on morphological characters of insects. Total number of fruit fly species will be calculated species wise. The generated data will be used to develop population dynamics of fruit flies species over a period of two years.

3) Studying the molecular diversity of fruit fly *B. zonata* populations in the cox 1 region of mitochondrial DNA

The fruit flies collected during survey will be used for DNA extraction by CTAB method. The extracted DNA will be subjected for Spectrophotometer for qualitative and quantitative analysis and will be stored in -20 ^{o}C for further use. The good quality DNA of *B zonata* collected from different districts will be used for amplifying the cox1 region of mitochondria DNA with specific primers by PCR technique (1). Five DNA samples from each district will be used for amplification of cox 1 region in mitochondrial DNA. The amplified DNA fragments will be eluated from gel, subjected to sequencing and followed by broad range of bioinformatics tool (BLAST, Clustal OMEGA, and barcoding platform) to understand the variations among the genes and obtained curated sequences will be submitted to gene bank.

4. To study the DNA Barcoding of fruit fly *B. zonata*

Further the sequences of cox 1 will be used for barcoding with other species and genera. Phylogenetic and evolutionary relationship of mitochondrial genome of *Bactrocera zonata*, is

compared to the homologous sequences of other tephritids through bioinformatics tools. Study is planned for three year.

Timeline

First Year :

1) Methyl eugenol traps will be installed in different districts of northern Karnataka and a month later fruit flies trapped in the container will be collected, brought to laboratory. The collected fruit flies will be used for DNA extraction studies.
2) Simultaneously fruit fly traps will be installed in fruit crops in Vijayapura, Riachur and Yadgiri districts to assess the species complexity and population dynamics of fruit fly *B. zonata*. This study will be continued for two years

Second Year :

1) The study on population dynamics of fruit flies will be continued even in second year also.
2) The fruit flies collected during survey will be used for DNA extraction stored in -20 ^{o}C for further use.
3) The cox1 region of mitochondria DNA will be amplified with specific primers by PCR technique.
4) The amplified fragments will be subjected to gene sequencing. The amplified gene sequences will be used for comparative analysis using tools like BLAST, Clustal MEGA, and NGS platform to understand the variations among the genes.
5) The obtained curated sequences will be submitted to gene bank.

Third Year :

1) Molecular diversity of fruit flies will be continued in third year also.
2) Further the sequences of cox 1 will be used for barcoding with other species and genera.
3) Phylogenetic and evolutionary relationship of mitochondrial genome of *Bactrocera zonata*, will be compared to the homologous sequences of other tephritids through bioinformatics tools.

Sl No	Objectives	First Year	Second Year	Third Year
1	Survey for the collection of *B. zonata* in different parts of Northern Karnataka	⟷		
2	Studying the population dynamics of *B. zonata* throughout the year	⟷	⟷	
3	Studying the Molecular diversity of *B. zonata* in the cox 1 and nad 1 region of mitochondrial DNA		⟷	⟷
4	To study the barcoding of *B. zonata*			⟷

	species with other species			

Time plan for Research work

Significance of study

Molecular diversity study of fruit flies is first of its kind in Karnataka more particularly in northern Karnataka. In Karnataka more research was carried out on *B. dorsalis* rather than *B.zonata*. Northern Karnataka is located in dry zone of Karnataka, where high temperature and dry weather prevails. The adverse climatic factors may affect the species diversity of fruit flies and their abundance. Even there could be existence of cryptic species. This information can be obtained from this study. Molecular diversity would certainly help us to understand the genetic structure, genetic variation among the population of *B. zonata*. Further the gene sequence could be used to develop barcode for accurate identification.

Expected outcome :

The study would provide the information about the genetic variation and presence of any cryptic species in the *B. zonata* population of northern Karnataka. This intern helps us for framing the management strategies effectively.

Reference

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